Remarks and Arguments

Claims 62-63, 65-66, 70-73, 75-79, 82, 97-90, 99-100, 102-110, 113, 118-125, 131 and 133 are pending. Claims 62, 70, 90, 102, 131 and 133 have been amended without prejudice or disclaimer of additional subject matter. Applicants reserve the right to present additional claims in one or more continuation or divisional applications.

Applicants wish to thank Examiner Ton and Examiner Paras for the courtesy of their interview with Dr. David Ayares and Amy Dandro and Applicant's representatives Susanne Hollinger and Becky Kaufman on May 14. Applicants have drafted the response below based on their understanding of the discussion and the requirements that were identified by the Examiners to further the prosecution of this case.

Rejection under 35 U.S.C. §112, first paragraph, written description

The Examiner has rejected claims 70-73 and 102-105 under 35 U.S.C §112, first paragraph as adding matter for the recitation of placing "a promoter adjacent to an endogenous gene" in the nuclear genome. Applicants have amended claims 70 and 102 to recite placement of a transgene adjacent to an endogenous promoter. This amendment is consistent with the Examiner's assertion that the disclosure "teaches placing an exogenous gene driven by an endogenous promoter," and finds support throughout the specification, but for example in paragraph 0200. Applicants believe this amendment overcomes the rejection as to added matter and ask for withdrawal of this rejection.

Rejection under 35 U.S.C. §112, second paragraph

The Examiner has rejected claims 62, 90, 131 and 133 and their dependents as indefinite for the recitation in step (a) of modifying the nuclear genome of a somatic cell with a normal karyotype at an endogenous locus. To clarify the claims, the independent claims have been amended to specify that the *genome* has a normal karyotype. The Examiner has also rejected claims 70 and 102 as unclear for the recitation of placing a promoter adjacent to the endogenous gene. These claims have been amended as noted above.

Applicants believe these amendments address the Examiner's rejection. However, should the Examiner's rejections on the above points remain as to the amended claims, she is asked to contact the undersigned to discuss appropriate amendments to clarify the subject matter.

Rejection under 35 U.S.C. §112, first paragraph, enablement

The Examiner has rejected all pending claims under 35 U.S.C. §112 as lacking enablement for methods of producing a non-human transgenic comprising:

1) the breath of genetic modifications of insertion of a promoter adjacent to an endogenous gene (based on rejected claims 70-73 and 102-105, which have now been corrected);
2) utilizing oocytes that have not been enucleated;
3) utilizing surrogate mothers other than those of the same species; and
4) in vitro targeted modification of any somatic cell other than fibroblasts.

Point (1) - Promoter insertion

As noted in the interview, claims 70-73 and 102-105 have been amended to recite placement of a gene adjacent to an endogenous promoter. Applicants believe that this amendment addresses the Examiner's rejection as to point (2).

Point (2) - Enucleation

Applicants have also amended the independent claims to refer specifically to recite transfer to an oocyte, two cell embryo or zygote all of which have been enucleated. This amendment finds support throughout the specification, but specifically for example in paragraphs 0031, 0045 and 0095-0097. Applicants believe this amendment overcomes the Examiner's rejection as to point (3).

Point (3) - Surrogates

The Examiner has also asserted that the claims are overly broad in including any surrogate of any species. In discussion with Examiner's Ton and Paras, it became clear

that the term "species" is not being used by the USPTO in its taxonomic meaning. Applicants have not asserted that *all* species can be used to develop the animal to term, but *do* assert that species within a given genus can be used interchangeably. In support of this contention, Applicants provide Kraemer, D.C. (1983) "Intra- and Interspecific Embryo Transfer" *J. Experiment. Zool.* 228:363-371, which is an early review detailing that intra-genus transfers result in viable offspring while inter-genus transfers are much more variable. These results have born out through subsequent work (see for example Haitch (1985) ""Follow-up on the News; Ox born to Cow" *The New York Times*; Santiago-Moreno et al. (2001) "Procedure for Successful interspecific embryo transfer from Mouflon (*Ovis gmelini musimon*) to Spanish Merino Sheep (*Ovis aries*)" *J. Zoo Wildlife Med.* 32:336-341; and Gomez et al. (2006) "Nuclear Transfer in Cats and its Application" *Theriogenology* 66:72-81).

The Examiner has cited to the specification as noting that sheep surrogates can be recipients for bovine, ovine or porcine species. It should be noted that the passage cited to by the Examiner relates to a particular *culturing* process that can be used to grow the embryo for a short time *in vivo*. The passage does not refer to the final surrogate that is used to develop the embryo to term, as the same paragraph states that the embryo is cultured until it is suitable for transfer to a "final surrogate recipient…in order to be grown to term" (see para 0107). Although Applicants believe that the claims already addressed this issue, in an attempt to clarify that the claimed surrogate is the one which will allow the embryo to be grown to term, the claims have been amended to mirror the language in paragraph 0107 and refer to a 'final' surrogate which is a suitable host for the animal to be grown to term.

Applicants believe this amendment addresses the Examiner's rejection on this issue and request withdrawal of this rejection. Should the Examiner feel additional amendments are required to address this issue, she is asked to contact the undersigned representative on this matter.

Point (4) - Targeting of somatic cells

As discussed during the interview, Applicants appreciate that the Examiner considers the claims enabled for the scope of fibroblasts. However, Applicants believe that the Examiner is mistaken in limiting the scope of enablement to fibroblasts rather than a broader set of somatic cells.

First, as discussed in the interview and is likely not apparent to the Examiner based on the references cited, the term 'fibroblast' when used in the context of gene targeting art is a term of art that relates to the general morphology of the cells, rather than to a characterized lineage. Therefore, when the Examiner asserts that 'fibroblasts' are enabled, the cells referred to are actually only morphologically fibroblast-like.

The present inventors discovered, contrary to what was assumed in the prior art, that it was possible to selectively target genetic modifications in somatic cells and use them for nuclear transfer. Applicants do not contest that certain types of somatic cells may not be useful in this process. However, the key feature to the cells that are used in this process, as noted in the present specification and detailed in the post-filing art that built on this invention, is that the cells have an extended lifespan sufficient to be useful in genetic modification and be viable for nuclear transfer before senescence. In particular, steps to be taken to improve the utility of these cells for nuclear transfer are detailed, for example in paragraphs 0168-0172 of the specification, as well as 0029-0042, 0116-0141, 0173-0177 and in the provided Examples. To clarify the claims, these have been amended to recite that the nuclear donor is a fibroblast or "other somatic cell that has a sufficient lifespan to be useful for genetic modification." This amendment finds support, for example, in para 0119 which describes the limited lifespan of the cells in culture and para 0168, which describes the problems of limited lifespan of somatic cells.

However, Applicants believe that the Examiner is mistaken in limiting the scope of enablement to fibroblasts rather than a broader set of somatic cells. First, as discussed in the interview, the term 'fibroblast' when used in the context of gene targeting art is a term of art that relates to the general morphology of the cells, rather than to a characterized lineage. The art would not place a premium on characterizing the lineage of a given cell, as it would not be critical or even important in the field. Instead, the gene targeting art - including those references cited by the Examiner- use the term

"fibroblast" to refer to any cell that has the general appearance of a fibroblast, even when that may not be accurate were the cell to be further characterized. The fact that the art has generally not cared about whether the cell at issue are in fact fibroblasts, or only fibroblast-like, may not be readily apparent to the Examiner simply because the references are unlikely to address the distinction, given that the point is largely irrelevant to the work at hand. The Examiner has requested that Applicants provide support for the assertion that the cells that are discussed in the post-filing art and considered enabled by the Examiner are not necessarily fibroblast in origin. As discussed, the primary support for this assertion is a *lack* of characterization, rather than a definitive characterization of such cells. This is due to the fact that the art generally has not cared whether the cells are indeed fibroblasts or just fibroblast-like in their growth characteristics.

The art has in part neglected to identify cell lineage because the culture conditions in which the cells are grown can induce a fibroblast-like phenotype in a variety of cells. This process is termed an "epithelial to mesenchymal transition" (EMT), and is reviewed in detail in Kalluri and Neilson (2003) *J. Clin. Invest.* 112:1776-1784. As noted by Kalluri and Neilson, EMT is a central mechanism for diversifying cells in complex tissues and in particular is responsible for changing cells such as epithelial cells into fibroblasts. Certain factors, notably addition of fibroblast growth factor (FGF), which is typically included in cell growth media, can induce EMT. Therefore, it is likely that many of the cells that resemble fibroblasts in culture did not originate as such and instead carry markers for their cell type of origin. The art recognizes this complexity in identifying the origin of cultured cells. For example, in their genetic analysis of fibroblast cells, Chang et al. note that "[i]t is now clear that the cells we traditionally call fibroblasts comprise a diverse class of distinct differentiated cell types…" (see Chang, et al. (2002) *PNAS* 99:12877-12882, page 12882).

Applicants note that methods of preparing cells for nuclear transfer generally involve taking a tissue (or entire fetus), cutting this tissues into small pieces and dispersing the cells. These dispersed cells are then grown *in vitro* and, after adherence, are referred to as "fibroblasts." Applicants are aware of only one study in this field in which any further investigation as to the cellular identity of the cells was conducted

(Onishi, et al. (2000) *Science* 289:1188-1190), in which PCR analysis revealed the cells to be negative for cytokeratin and embryonic antigen-1 but positive for vimentin[1]. Remaining studies, including Lai, et al. (2002) *Science* 295:1089-1092 and Dai, et al. (2002) *Nature Biotech.* 20:251-255 describe the culturing of cells without analysis of the cell type or identification by anything other than morphological characteristics. Denning et al. (2001) *Cloning Stem Cells* 3:221-231 in fact specifically note that the "fibroblast cells" discussed throughout the paper are identified because "[b]ased on morphological appearance, fibroblastic cells predominated after about the third passage…" Other investigators, also termed certain cells as "fetal fibroblasts" even though these are merely described as "fibroblast-like cells." For example, Wilmut et al. described the cloning of sheep using what is termed in Figure 1 a population of "fetal fibroblasts (BLWF1)" even though, in the description of the methods of isolation of the cells, the authors described these same cells merely as "fibroblast-like" (see Wilmut et al. (1997) *Nature* 385:810-813, compare Figure 1b to page 813, top left).

Lastly, Applicants note that the Examiner was mistaken in her understanding of the technology in Example 5 and point out that the present specification actually provides examples of targeting both fibroblast *and non-fibroblast* cells. As discussed in the interview, the Examiner was mistaken in identifying Example 5, which utilizes a "promoter trap" vector to modify mammary cells, as working by "random" integration. As discussed, Example 5 shows the targeted integration in a somatic cell from a different set of tissues. The vector described in Example 5 is designed such that expression of the selectable marker only occurs when targeted integration (into the endogenous promoter locus) occurs. Therefore, promoter trapping enriches for targeted events.

As noted above, the term "fibroblast" is an art-accepted term related to the general morphological characteristics rather than lineage of certain cells. The Examiner's assertion that the only cells that are enabled are fibroblast cells should thus be qualified, in that none of the post-filing art relied on by the Examiner actually characterizes the cells. As noted in both the present specification and subsequent art, a required feature for these cells is not what lineage they are derived from, but that their lifespan is similar to

[1] Applicants note that vimentin is a marker for identifying cell types of mesenchymal origin, not for identifying fibroblasts specifically.

that disclosed for the cells used in the present specification. Furthermore, the present application actually describes methods of targeting two different kinds of somatic cells, not just one as originally determined by the Examiner. Therefore, to the extent that the Examiner accepts enablement of fibroblasts, the cells identified by the Examiner should not be limited by their origin, but rather by their secondary characteristics that are necessary for the process of the invention. Applicants respectfully request withdrawal of this rejection.

Rejections under 35 U.S.C. §102(b)/103(a)

The Examiner has rejected claims 62-63, 65-66, 75-76, 82, 87-90, 99-100, 106, 113, 118-122, 131 and 133 under 35 U.S.C §102(b) as anticipated or alternatively under 35 U.S.C. §103(a) as obvious over Campbell, et al. (WO 97/07669). The Examiner contends that Campbell teaches methods of producing transgenic animals via nuclear transfer and that transgenic animals can be produced by their methods.

Applicants do not dispute that Campbell was the first disclosure proving that a viable animal could be developed from a somatic cell nucleus using nuclear transfer. As discussed in the interview, the key problem with Campbell is that one of skill in the art would have interpreted the discussion of genetically modified animals as merely a hoped for result, based on the overall teaching in the art as well as the lack of any additional results in Campbell that would support targeted transgenic animals from somatic cells. There is nothing in Campbell that dispel the substantial uncertainty that one in the art would have had as to whether any techniques could be used to make a targeted transgenic animal using somatic cell nuclear transfer, let alone any specific methods that would have enabled anyone to actually produce such animals with even a minimal expectation of success. The present invention, in contrast, proved that the production of live animals with a targeted genetic modification were viable and provided specific techniques to lead to successful animal production.

Substantial Uncertainty/lack of success

As the Examiner has herself identified, there was a strong belief throughout the art, both before and after Campbell, that somatic cells that had been grown in culture for

the significant periods that were needed to identify a targeted modification were no longer useful for producing viable animals through nuclear transfer. To be anticipating, a prior art reference must enable the claimed invention (see e.g. *Elan Pharms., Inc. v Mayo Found.*, 346 F.3d 1051 (Fed. Circ. 2003)). If the disclosure is merely a starting point for experimentation and a substantial uncertainty remains as to the success of the process, the reference is not anticipating (see e.g. *Dewey & Almy Chem. Co. v. Mimex Co.*, 124 F.2d 986 (2d Cir. 1942)).

As noted in the specification, it was well known that the ability to target versus randomly integrate into somatic cells was much lower than into embryonic stem cells (see para 0117, Arbones, et al. (1994) *Nat. Genet.* 6:90-97), that primary cells have a lower frequency of homologous recombination than immortalized cells (see para 0118, Finn, et al. (1989) *Mol. Cell. Biol.* 9:4009-4017 and Thyagarajan et al. (1996) *Nuc. Acids Rsch.* 24:4084-4091). This perceived difficulty in the prior art is highlighted in Suraokar and Bradley (2000) *Nature* 405:1004-1005, which is a comment on the publication of the present invention. In particular, Suraokar and Bradley comment on the contribution that the cloning of Dolly (i.e. the disclosure in Campbell) had on the possibility for making transgenic animals. As noted in Suraokar and Bradley, Campbell "revived" the hope that livestock could be genetically modified, *however,* the authors note that "[u]ntil now, no one had shown that it would be possible to specifically modify endogenous genes by cloning." Until the date of the present invention therefore, skilled artisans were still looking for proof that the present invention of gene targeting and nuclear transfer "would be possible."

The prior art reflected that the skilled artisan would have believed that additional techniques would be needed to produce viable, genetically targeted animals. As noted in the declaration of Dr. Azim Surani, provided in the parent case US 09/475674 in 2001 and re-attached hereto, several factors, including the process of genomic imprinting were considered major obstacles to the use of genetically targeted somatic cells before the present filing. As noted in Porter, et al. (1997) *Transplant.* 64:1227-1235, targeting in somatic cells was not yet a realistic option. Specifically, these authors note that "[m]ajor improvements in this and/or the efficiency of gene targeting itself will be required before

the use of gene-targeted cells in autologous transplantation can be seriously contemplated" (page 1229). Even shortly after the filing of the present case, authors such as Piedrahita note that techniques available at the time were not likely to lead to success targeted genetic modifications in somatic cells that could then be used for nuclear transfer, and that "in the future" techniques may be useful for producing such targeted animals (see Piedrahita (2000) *Transgenic Research* 9:261–262).

Campbell does not provide any techniques to overcome these art-recognized difficulties. Campbell notes that "transgenic animals may be produced from genetically altered donor cells, that there are advantages that could be seen by using the nuclear transfer techniques to develop genetically modified animals, and that there are numerous techniques that can be used for genetically modifying cells (see statements on pages 5-7 and 19-20 of Campbell). There are no statements that address techniques that would have been considered by one of skill in the art to overcome the problems of low targeting frequency and early senescence that were well known in the prior art.

The passages in Campbell that relate at all to genetic modification merely refer to the fact that successful nuclear transfer is a step towards the successful generation of genetically modified animals. The Applicants do not dispute this assertion. It is indeed true that successful nuclear transfer was a step towards the development of a genetically modified animal. What Applicants *do* dispute is that a reference that recites the mere desirability of a genetically modified animal is sufficient to enable a targeted genetic modification of a somatic cell and its subsequent use in nuclear transfer. As nothing in Campbell provided one of skill in the art with a technique that would be expected to be useful without undue experimentation, Campbell does not destroy the novelty of the pending claims.

Non-obviousness

Campbell fails as a reference under 35 U.S.C. §103(a) for much the same reason as it fails under 35 U.S.C. §102. The reference simply provides no more than a hoped for result in a field in which the hoped for result was considered to be outside of the ability of the ordinarily skilled artisan. As discussed above, because primary cells have a limited

lifespan in culture, their competence as nuclear donors was expected to decrease over time. Specifically, it was expected that prolonged culture would cause detrimental changes in the somatic cell nucleus including mutations in the genome and epigenetic changes that would make any embryos derived from such cells non-viable. Therefore, before the present invention, it was not believed that there were any available techniques that would have allowed the targeted genetic modification and subsequent selection of donor somatic cells sufficient to produce viable animals.

As the Examiner is well aware, an "essential component" of the obviousness determination is the analysis of secondary indicia of nonobviousness (see *Graham v. John Deere Co. of Kan. City*, 383 U.S. 1), which include among others: a long felt need, skepticism of skilled persons prior to the invention, and copying of the invention.

As noted in references including Campbell itself, the desirability of targeted genetically modified large animals was well documented in the prior art. However, Applicants note that even after Campbell's filing, it took over four years to develop the presently claimed techniques to produce viable, genetically targeted animals using somatic cell nuclear transfer.

Furthermore, as noted above, it is well documented that the skilled artisan prior to the present filing would have expected known techniques to fail in the development of viable animals. The fact that an ordinary artisan would have found the success surprising is itself unexpected, as noted by the Federal Circuit in *In re Soni,* 54 F.3d 746, 750 (Fed. Circ. 1995).

In addition, Applicants note that the present invention is the foundational discovery that has led to the development of targeted animals for potential use in xenotransplantation or medical device development. To the Applicant's knowledge, *all* later authors publishing on genetically modified large animals have followed on the techniques described in this application. Furthermore, the paper in which the present invention was published in the scientific literature, McCreath, et al. (2000) *Nature* 405:1066-1069 has been cited at least 381 times by other scientific publications.

The long felt need to develop targeted genetically modified large animals, coupled with the lack of expectation of success using prior art techniques and the proven

influence of the present invention in this field all support the non-obviousness of the present invention over Campbell.

The Examiner has also rejected claims 76-79, 107-110 and 12-124 under 35 U.S.C. §103(a) as obvious over Campbell in view of dApice, et al. (U.S. Patent No. 5,849,991). As noted above, Campbell does not anticipate or render obvious the claims because it provides merely a hoped for result with no proof that would have led a skilled artisan to believe that any available methods could overcome the art-recognized hurdles that led to an expectation of failure before the present invention. d'Apice merely provides homozygous *mice* that lacked a particular gene, α-1,3-galactosyltransferase that were made using embryonic stem cells, and noted that animals lacking this gene would potentially be useful in reducing hyperacute rejection. As to large animals, for which ES cells were not available, there is no additional teaching in d'Apice that would suggest that a technique had been developed to overcome the hurdle of somatic cell senescence. Therefore, a skilled artisan reading Campbell alone or in combination with d'Apice would not believe that a viable animal with a targeted genetic event could be produced using the techniques as recited in the amended claims.

Similarly, the Examiner has rejected claims 70, 73, 77, 102, 105, 107, 108 and 125 under 35 U.S.C.§103(a) over Campbell in view of Kucherlapati et al. (WO 94/02602). As noted above, Campbell itself does not anticipate or render obvious the claims. Kucherlapati, much like d'Apice, provides merely techniques that could be used in animals in which embryonic stem cells could be used to make transgenic animals. This technique was well known for mice for many years prior to either the present invention or the disclosure of Campbell. However, these techniques are inapplicable to the present invention, which specifically relates to the use of somatic cells and nuclear transfer, *not embryonic stem cell technology.* As noted in the present specification and in Campbell, a major obstacle in production of any cloned large animals has traditionally been that the embryonic stem cells, which are so useful in producing modified mice, are generally not available. Therefore, Kucherlapati provides no additional support to

Campbell for the production of *any* targeted transgenic animals. There is no doubt that the art felt a strong desire to make targeted genetic modifications in large animals. However until the present invention no one of any skill in the art expected that any available techniques could be successfully used to produce viable animals.

None of the cited references, alone or in combination overcome the art-recognized difficulties and lack of expectation of success in producing viable animals with targeted genetic modifications from somatic cell nuclear transfer. The present invention was the first in the world to produce such animals, which had long been desired but never made. Applicants respectfully request withdrawal of these rejections.

No further fees are believed to be due in connection with this response. However, the Commissioner is hereby authorized to charge any underpayment or credit any overpayment of fees to Deposit Account No. 11-0980.

Respectfully submitted,

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